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Synthesis and biological evaluation of enantiomerically pure pyrrolyl-oxazolidinones as a new class of potent and selective monoamine oxidase type A inhibitors

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Abstract

Due to the key role played by monoamine oxidases (MAOs) in the metabolism of neurotransmitters, MAO inhibitors (MAOIs) represent an useful tool for the treatment of several neurological diseases. Among selective MAOIs, MAO-A inhibitors (e.g. clorgyline) are used as antidepressant and antianxiety drugs and are claimed to protect neuronal cells against apoptosis, and selective MAO-B inhibitors (e.g. L-deprenyl) can be used in the treatment of Parkinson's disease either alone or in combination with L-DOPA. However, they engender covalent bonds with the active site of the enzyme and induce irreversible inhibition; moreover, they tend to lose their initial selectivity at high dosages or with repeated administrations. Phenyloxazolidinones belong to third-generation-MAOIs, characterized by a selective and reversible inhibition of the enzyme. Among these molecules, the most representative are toloxatone and befloxatone, two selective and reversible MAO-A inhibitors used in therapy as antidepressant drugs. Going on our searches on CNS potentially active compounds containing a pyrrole moiety we prepared 3-(1*H*-pyrrol-1-yl)-2-oxazolidinones (**1**) and isomeric 3-(1*H*-pyrrol-2-and -3-yl)-2-oxazolidinones (**2** and **3**) as anti-MAO agents. Such derivatives resulted selective and reversible MAO-A inhibitors. The most potent compound is (*R*)-5-methoxymethyl-3-(1*H*-pyrrol-1-yl)-2-oxazolidinone (**1b**), endowed with very high potency ($K_{iMAO\text{-}A} = 4.9$ nM) and A-selectivity (A-selectivity = 10,200, about 116-fold greater than that of befloxatone).
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Keywords: MAO; MAO inhibitors; Oxazolidinones; Antimycobacterial agents

1. Introduction

Monoamine oxidase (MAO, EC 1.4.3.4) is a flavoprotein located at the outer membranes of mitochondria in neuronal, glial, and other cells. It catalyses the oxidative deamination of monoamine neurotransmitters such as serotonin (5-hydroxytryptamine, 5-HT), norepinephrine, and dopamine, and appears to play important roles in several psychiatric and neurological disorders [1,2] (Figs. 1 and 2). In addition, it is also responsible for the biotransformation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) into 1-methyl-4-phenylpyridinium (MPP^+), a Parkinson producing neurotoxin [3–5]. Recently, it has been shown that MAO contributes to the apoptotic process because inhibition of MAO activity suppresses cell death [6].

MAO exists in two forms, namely, MAO-A and MAO-B, distinguishable by their molecular cloning, substrate and inhibitor selectivity, and tissue distribution [7–10]. MAO-A preferentially oxidizes serotonin and is irreversibly inhibited by low concentrations of clorgyline [8]. MAO-B preferentially oxidizes β-phenylethylamine (PEA) and benzylamine, and it is irreversibly inactivated by low concentrations of L-deprenyl [11]. Dopamine, tyramine, and tryptamine are common

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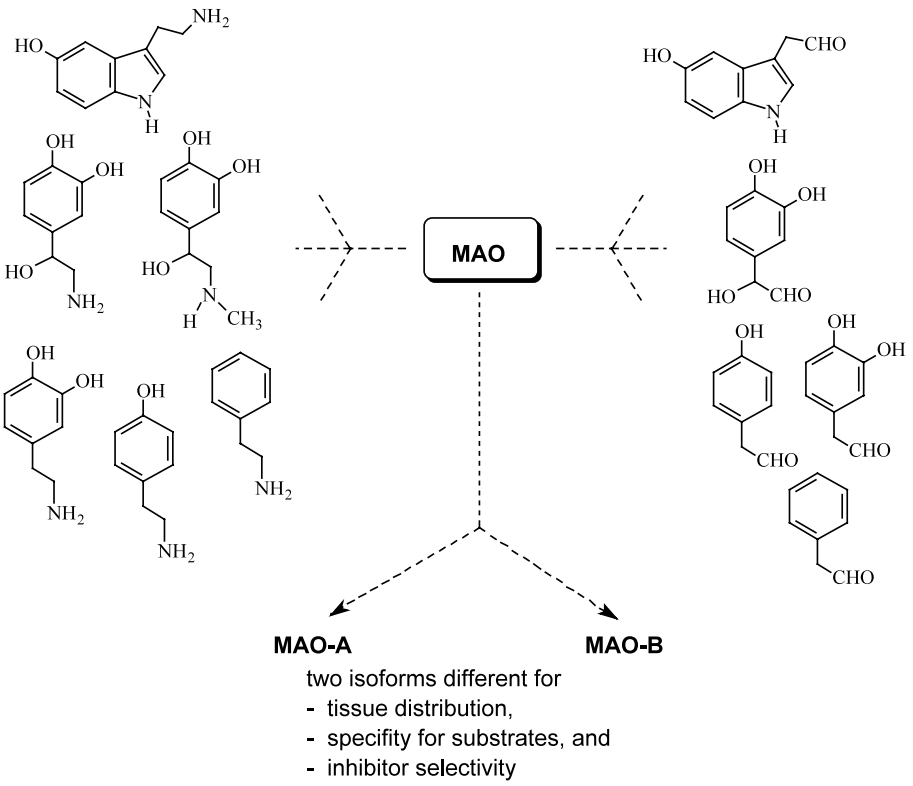


Fig. 1. Oxidative deamination catalysed by monoamine oxidase (MAO) enzymes.

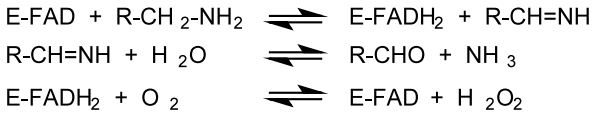


Fig. 2. Simplified scheme of the reaction catalyzed by MAOs.

substrates for both MAOs. MAO-A and MAO-B consist of 527 and 520 amino acids, respectively, and have a 70% amino acid identity [7]. Each isoenzyme has a FAD moiety covalently linked to a cysteine residue, Cys406 (MAO-A) or Cys397 (MAO-B), through an 8α-cysteinyl-riboflavin (Fig. 3) [12–15]. They are closely linked to the X-chromosome and exibit identical exon–intron organization, and they are probably derived from the duplication of a common ancestral gene [16]. When the MAO-A gene is deficient in humans [17] and mice [18] higher 5-HT and norepinephrine levels and a phenotype characterized by increased aggressive behavior is observed. Disruption of the MAO-B gene in mice results in increased PEA but not 5-HT, norepinephrine, or dopamine and confers a resistance to the Parkinsonism-inducing toxin MPTP [5] (Tables 1 and 2).

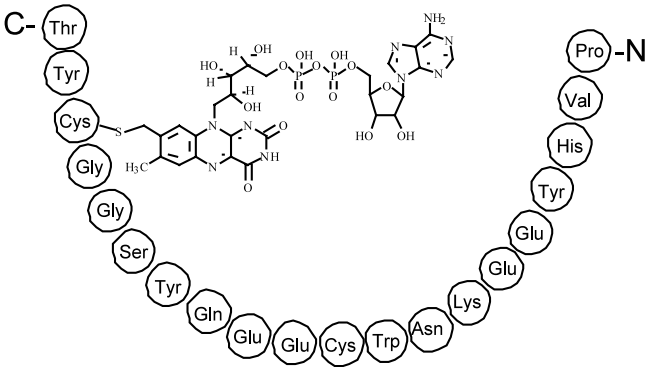


Fig. 3. Chemical structure of flavin-adenin diphosphate (FAD) covalently bound to a cysteine of the FAD-peptide in human MAO-A and MAO-B.

Due to the key role played by the two MAO forms in the metabolism of monoamine neurotransmitters, MAO inhibitors (MAOIs) can represent an useful tool for treatment of several neurological diseases. MAOIs such as phenelzine, tranylcypromine and isocarboxazid were introduced in psychiatry during the late 1950s and were the first of many antidepressants (ADs) to enter the clinical medicine (Fig. 4). However, their use became limited, to the profit of tricyclic ADs, as they were found to induce severe food (tyramine rich/cheese effect) and drug interactions.

A second generation of MAOIs emerged with the discovery of selective inhibitors of the A and B forms of the enzyme. Since 5-HT and norepinephrine are preferentially deaminated by MAO-A and since dopamine and tyramine are substrates of both forms, selective MAOIs opened new frontiers for the use of MAOIs as antidepressants (Fig. 4). They should result more selective in terms of both target symptoms and adverse reactions than the earlier drugs and, compared with

Table 1
Concentration of MAO-A and MAO-B in membrane preparations from various brain areas, extracerebral tissues and cell lines

	MAO-A (pmol/mg)	MAO-B (pmol/mg)	MAO-A/MAO-B ratio
Human			
Frontal cortex	2.6±0.4	7.1±2.0	0.37
Hypothalamus	5.2±1.0	17.8±5.1	0.29
Substantia nigra	2.4±0.2	13.3±3.0	0.18
Raphe	3.4±0.8	20.7±5.8	0.16
Hippocampus	3.4±0.8	20.7±5.8	0.16
Cerebellum	2.5±0.4	5.6±1.6	0.45
Placenta	101.7±36.5	0.8±0.3	126.00
Platelets	nd	6.9±0.3	
Hep G2 [a]	9.4±0.5	3.1±0.3	3.30
Rat			
Frontal cortex	12.2±0.4	2.9±0.3	4.20
Cerebellum	3.0±0.4	2.2±0.4	1.40
PC 12[b]	13.0±0.9	nd	
Liver	12.2±0.4	22.9±2.4	0.53

ND: not detected.
[a] Human hepatoma epithelial-like cell line.
[b] Rat pheochromocytoma cell line.

Table 2
Favoured substrates of cerebral MAO-A (A) and MAO-B (B)

Substrate	In vivo		In vitro
	Man	Rat	
5-Hydroxytryptamine	A	A	A
Norepinephrine	A (B)	A	A+B
Epinephrine	A	A	A+B
Dopamine	B (A)	A	A+B
2-Phenylethylamine	B	B	B
Tyramine[a]	A+B	A+B	A+B
MPTP[b]	B	B	B

[a] Exogenous tyramine is mainly metabolized by MAO-A in the intestinal tract.
[b] 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

tricyclic ADs, they appeared to have a wide spectrum of action that included the relief of anxiety disorders [19]. Among selective MAOIs, MAO-A inhibitors (e.g. clorgyline [8]) are used as antidepressant and antianxiety drugs and are claimed to protect neuronal cells against apoptosis [20], and selective MAO-B inhibitors (e.g. L-deprenyl [21]) can be used in the treatment of Parkinson's disease either alone or in combination with L-DOPA [22].

However, they engender covalent bonds with the active site of the enzyme and induce irreversible inhibition; moreover, second-generation-MAOIs tend to lose their initial selectivity at high dosages or with repeated administrations. This way of action produces many important limitations to the use of these compounds in therapy, such as central nervous effects (insomnia, irritability, agitation, hypomania, suppression of REM sleep), cardiovascular disfunctions (orthostatic hypotension), severe hypertensive reactions and sexual disturbances [23,24].

During the 1980s a third generation of MAOIs appeared: the reversible and selective inhibitors, which from theoretical considerations were expected to have higher selectivity over a wide dose range and during chronic use, thereby inducing minimal adverse side effects.

Reported reversible MAOIs belong to the morpholino [25], piperidino [26], 2-aminoethylcarboxamide [27], and 2-oxazolidinone series [28] (Fig. 5). Toloxatone (Humoryl®), a new antidepressant agent marketed in France in 1985, is the prototype of 3-phenyl-2-oxazolidinones, a class of MAOIs highly active mainly against the A isoform of the enzyme [29–32]. Chemical modifications performed on toloxatone have led to cimoxatone and, more recently, befloxatone, an anti-MAO agent active at nanomolar range and more A-selective than toloxatone (Fig. 5) [33,34]. However, such chemical manipulations have regarded the substituent(s) located on the phenyl ring at the N_3 position or at the C_5 methylene group of the 2-oxazolidinone ring, while little or no attention has been devoted to the replacement of phenyl with heteroaromatic rings [35].

Pursuing our searches on synthesis and biological evaluation of pyrrole-containing compounds active on central nervous system [36–39], we planned the preparation of pyrrole derivatives containing the 2-oxazolidinone nucleus linked at different position of the pyrrole ring. Three diverse series of pyrrolyl-oxazolidinones have been synthesized and tested as new anti-MAO agents: (i) 3-(1*H*-pyrrol-1-yl)-2-oxazolidinones; (ii) 3-(1*H*-pyrrol-2-yl)-2-oxazolidinones; and (iii) 3-(1*H*-pyrrol-3-yl)-2-oxazolidinones.

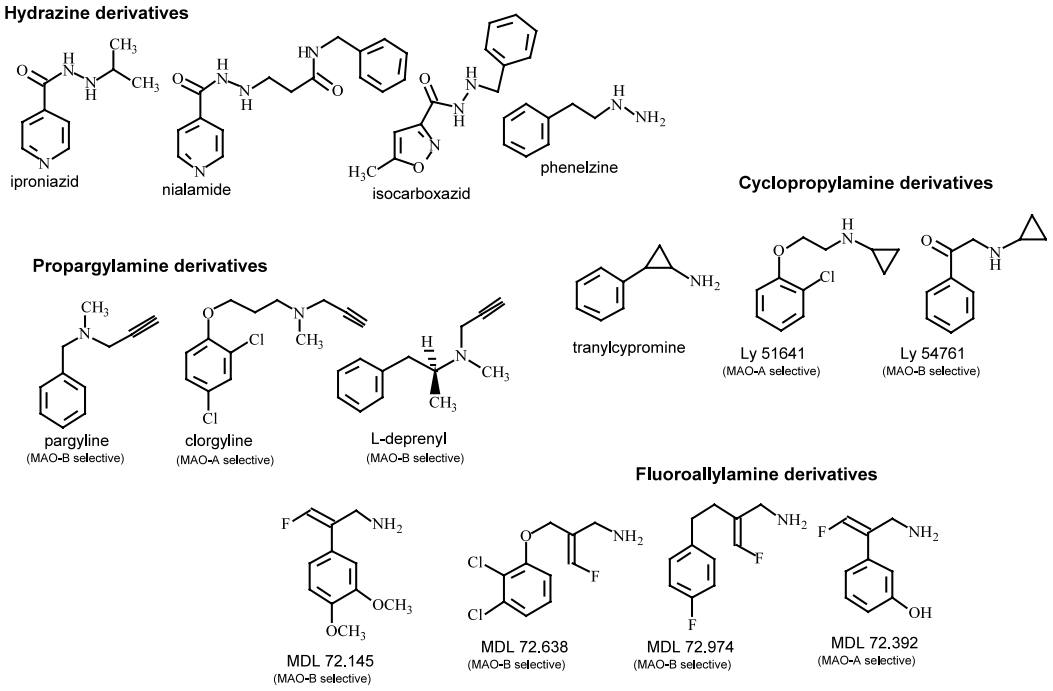


Fig. 4. Irreversible MAOIs belonging to different chemical classes.

2. Chemistry

2.1. 3-(1H-Pyrrol-1-yl)-2-oxazolidinones (1)

3-(1*H*-Pyrrol-1-yl)-2-oxazolidinones are characterized by the pyrrole moiety linked to the 2-oxazolidinone ring through a N–N linkage, and bear different substituents (i.e. hydroxy-, alkoxy-, azido-, alkylamino-, acyloxy-, acylamidomethyl, etc.) at the C_5 position of the oxazolidinone (Fig. 6). As a chiral center is aroused into the oxazolidinone moiety by the insertion of a C_5 substitutent, both the enatiomerically pure (*R* and *S*) series of compounds have been synthesized and tested.

The synthesis of 3-(1*H*-pyrrol-1-yl)-2-oxazolidinones starts from 1-(phenylmethoxy carbonylamino)-1*H*-pyrrole, which was prepared from benzyl carbazate and 2,5-dimethoxytetrahydrofuran in ethanol/acetic acid medium. After treatment with *n*-butyl lithium in hexanes at −70 °C, the lithiated intermediate reacted with enantiomerically pure glycidyl butyrate to furnish directly, after spontaneous hydrolysis of the butyrate function, the (*R*)-5- and (*S*)-5-hydroxymethyl-3-(1*H*-pyrrol-1-yl)-2-oxazolidinones **1a** and **1n**, depending on the *R*- or *S*-glycidyl butyrate used, respectively.

The alcohols were converted into the corresponding methanesulfonates with methanesulfonyl chloride and triethylamine, and these compounds were subjected to

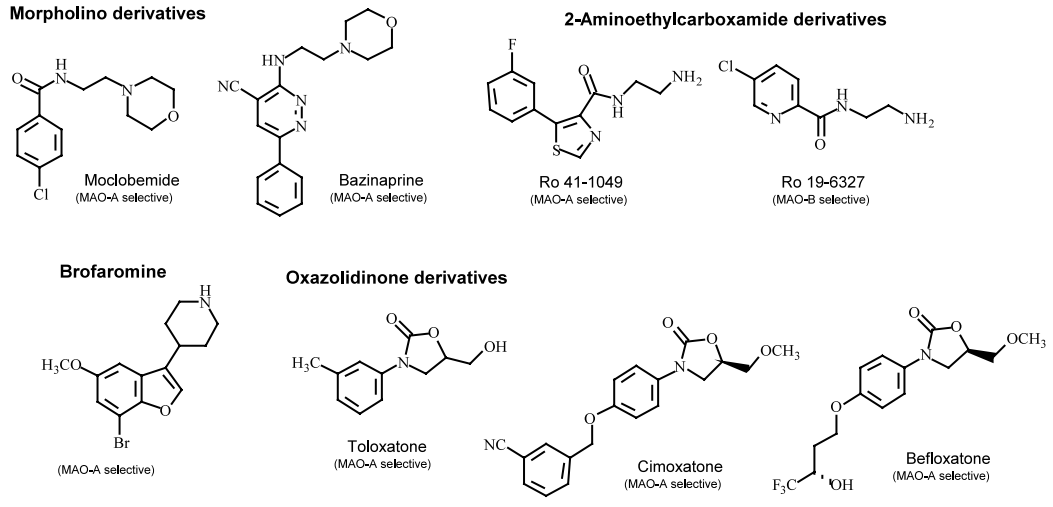


Fig. 5. Reversible and selective third-generation-MAOIs.

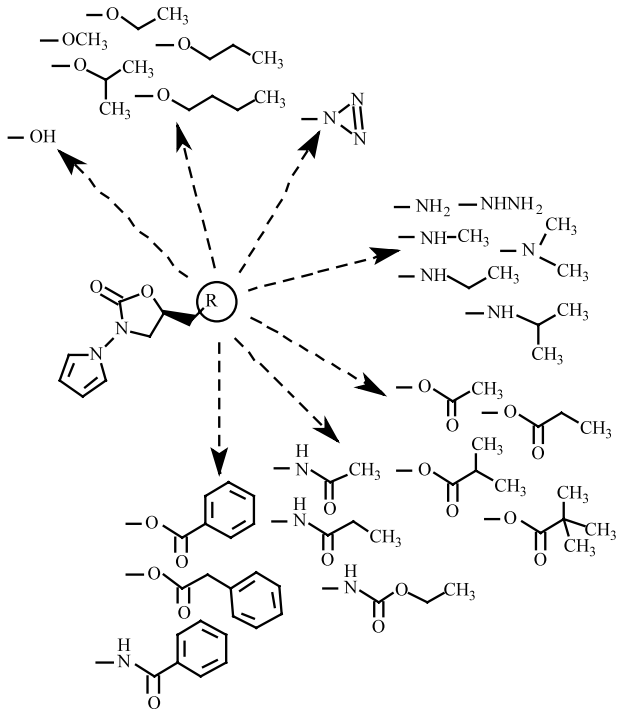


Fig. 6. 3-(1*H*-Pyrrol-1-yl)-2-oxazolidinones.

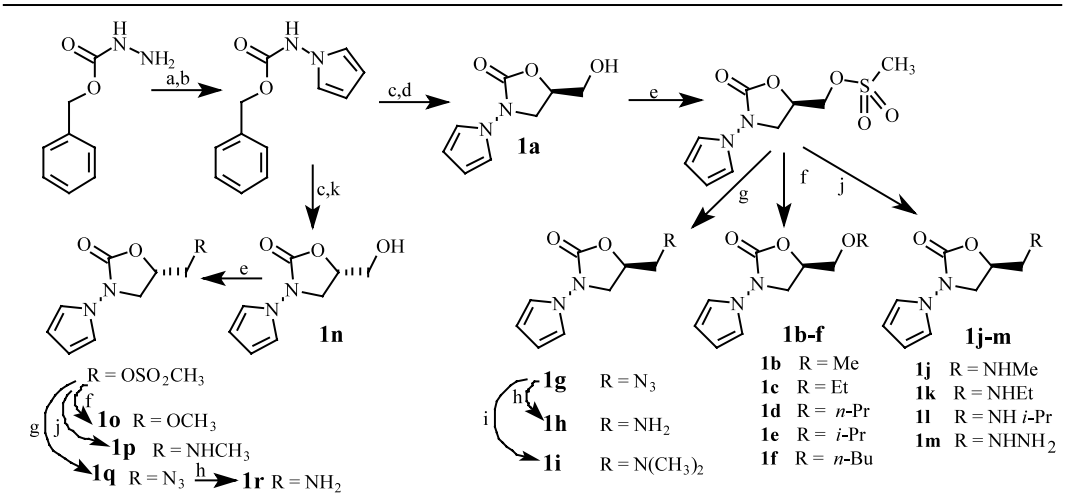
nucleophilic displacement by sodium alcohoxides, alkylamines, hydrazine and sodium azide to give **1b–g**, **j–q**. 5-Aminomethyl- and 5-dimethylaminomethyl derivates **1h**, **i**, **r** were obtained by catalitic reduction of the azidomethyl analogs with hydrogen (aminomethyl) or with hydrogen and formaldehyde in excess (dimethylaminomethyl) (Scheme 1). A sample of toloxatone was synthesized and used as reference drug in biological tests of derivatives **1**.

2.2. 3-(1*H*-Pyrrol-2- and -3-yl)-2-oxazolidinones **2** and **3**

The synthesis of derivatives **2** and **3** was performed starting from the appropriate 1-alkyl-1*H*-pyrrole-2- and 3-carboxylic acids, previously prepared by alkylation of 1*H*-pyrrole-2-carboxylic acid (commercially available) or by oxidation of 1-alkyl-1*H*-pyrrole-3-carboxaldehydes. The pyrrolecarboxylic acids were reacted with diphenylphosphonic azide, benzyl alcohol and triethylamine at high temperature to give after Curtius transposition the corresponding carbamoyl derivates, which were then cyclized with *n*-butyl lithium and *R*-glycidyl butyrate as previously reported to furnish the key intermediates 5-hydroxymethyl-1-alkyl-1*H*-pyrrol-2- and 3-yl-2-oxazolidinones. Subsequent transformations of these alcohols into the corresponding methanesulfonates, azides, amines, and alkylethers, were performed as described for the synthesis of the above reported series of pyrrole-containing oxazolidinones (Scheme 2).

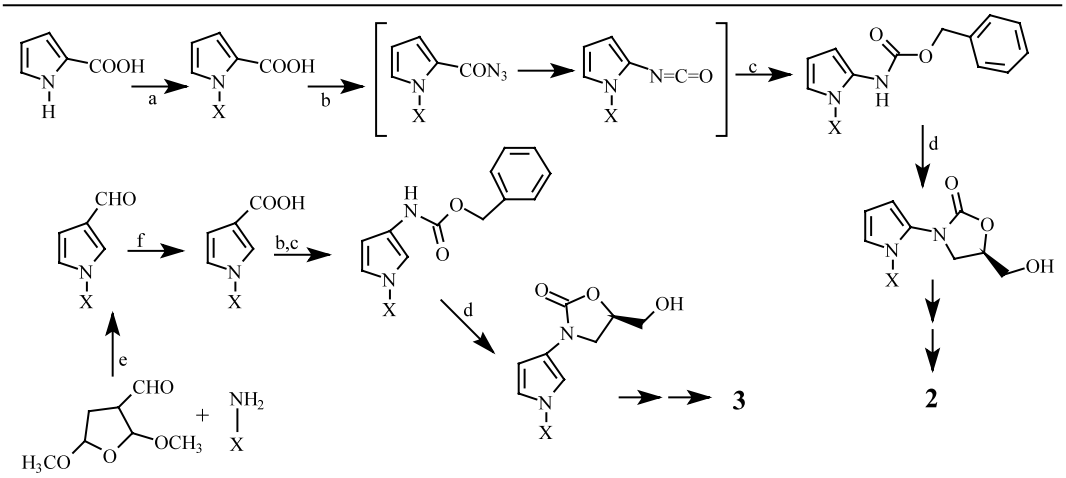
3. Biological evaluation: results and discussion

5-Substituted-3-(1*H*-pyrrolyl)-2-oxazolidinones **1–3** were evaluated for their ability to inhibit MAO-A and MAO-B, in comparison with toloxatone as reference drug. Bovine brain mitochondria were used as the enzyme source. Activities of MAO-A and MAO-B were determined by a fluorimetric method with kynuramine as substrate. The K_i values against the two isoenzymatic MAO forms and the A-selectivity (expressed as $K_{iMAO\text{-}B}/K_{iMAO\text{-}A}$ ratio) were determined.



[a] a: 2,5-Dimethoxytetrahydrofuran, AcOH, EtOH, Δ. b: AcOH, Δ. c: *n*-BuLi 2.5 M in hexanes, THF, - 78 °C. d: 1) *R*-glycidyl butyrate, 2) NH_4Cl, rt. e: CH_3SO_2Cl, Et_3N, CH_2Cl_2, rt. f: RONa, ROH, rt. g: NaN_3, DMF, 70 °C. h: H_2, Pd/C, rt. i: H_2, CH_2O, Pd/C, rt. j: RH, THF, 65 °C. k: 1) *S*-glycidyl butyrate, 2) NH_4Cl, rt.

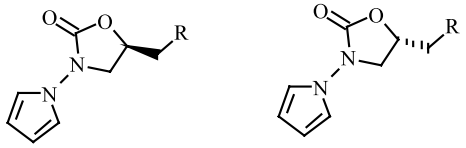
Scheme 1. Synthesis of 3-(1*H*-pyrrol-1-yl)-2-oxazolidinones (**1**).



[a] a: X-Hal, DMSO, KOH. b: $(PhO)_2PON_3$. c: $PhCH_2OH$, Δ. d: 1) *n*-BuLi 2.5 M in hexanes, - 78 °C; 2) *R*-glycidyl butyrate. e: AcOH. f: $AgNO_3$, NaOH.

Scheme 2. Synthesis of 3-(1*H*-pyrrol-2-and-3-yl)-2-oxazolidinones **2** and **3**.

Table 3
Monoamine oxidase inhibitory activity of compounds **1** [a]

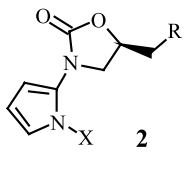


1a-m,s,t **1n-r**

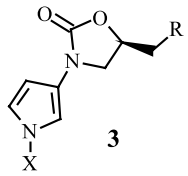
Compd.	C_5 configuration	R	K_i MAO-A (μM)	K_i MAO-B (μM)	A selectivity
1a	*R*	OH	0.09	9	100
1b	*R*	OCH_3	0.0049	50	10,200
1c	*R*	OCH_2CH_3	0.002	1.6	800
1d	*R*	$OCH_2CH_2CH_3$	0.014	4	286
1e	*R*	$OCH(CH_3)_2$	0.6	32	53
1f	*R*	$OCH_2CH_2CH_2CH_3$	0.97	10	10.3
1g	*R*	N_3	0.2	200	1000
1h	*S*	NH_2	53	4.4	0.08
1i	*S*	$N(CH_3)_2$	40	140	3.5
1j	*S*	$NHCH_3$	0.06	22	367
1k	*S*	$NHCH_2CH_3$	0.017	5	295
1l	*S*	$NHCH(CH_3)_2$	0.006	33	5500
1m	*S*	$NHNH_2$	0.9	25	28
1n	*S*	OH	1.2	2	1.7
1o	*S*	OCH_3	1	23	23
1p	*R*	$NHCH_3$	0.063	50	794
1q	*S*	N_3	2.5	24	9.6
1r	*R*	NH_2	1.2	34	28
1s	*R*	$OCOCH_3$	0.0065	2.7	415
1t	*R*	OCOPh	0.06	10	167
(*R*/*S*) toloxatone			0.38	15	39.5
(*R*,*R*) befloxatone [b]			0.0025	0.22	88

[a] Data represent mean values of at least three separate experiments.
[b] Ref. [33].

Table 4
Monoamine oxidase inhibitory activity of compounds **2**,**3** [a]



2



3

Compd.	X	R	K_i MAO-A (μM)	K_i MAO-B (μM)	A selectivity
2a	CH_3	OH	0.087	3.6	41.4
2b	CH_3	OCH_3	0.020	1	50
2c	CH_3	N_3	0.004	4	1000
2d	CH_3	NH_2	0.01	25	2500
2e	CH_2CH_3	OH	0.1	50	500
2f	CH_2CH_3	OCH_3	0.6	5.9	9.8
2g	CH_2CH_3	N_3	0.2	4	20
2h	CH_2CH_3	NH_2	0.2	45	225
2i	$CH_2CH{=}CH_2$	OH	0.43	5	11.6
2j	$CH_2CH{=}CH_2$	OCH_3	0.52	6.5	12.5
2k	$CH_2CH{=}CH_2$	N_3	0.35	5	14.3
2l	$CH_2CH{=}CH_2$	$NHCH_3$	0.04	5.5	137.5
2m	CH_2–Ph	OCH_3	50	70	1.4
2n	CH_2–Ph	N_3	30	77	2.6
2o	CH_2–Ph	NH_2	50	80	1.6
2p	CH_2–Ph	$NHCH_3$	0.14	0.5	3.6
3a	CH_3	OH	1	> 10	> 10
3b	CH_3	OCH_3	0.3	170	567
3c	CH_3	N_3	0.2	360	1800
3d	$CH_2CH{=}CH_2$	OH	0.35	625	1786
3e	$CH_2CH{=}CH_2$	$NHCH_3$	0.1	2	20
3f	SO_2–Ph	OH	0.4	0.1	0.25
(*R*/*S*) toloxatone			0.38	15	39.5
(*R*,*R*) befloxatone [b]			0.0025	0.22	88

[a] Data represent mean values of at least three separate experiments.
[b] Ref. [33].

The majority of compounds showed inhibitory activity against the A isoform of MAO enzyme higher than that exerted against the MAO-B. Furthermore, all derivatives displayed a reversible mode of action since dialysis for 24 h in a cold room against 0.1 M potassium phosphate buffer (pH 7.2) was able to restore 90–100% of the enzyme activity (Table 3).

Among compounds **1**, derivatives **1a**–**e**, **g**, **j**, **k**, **p**, **s**, **t** were the most active with concentration values of MAO-A inhibitory activity in the nanomolar range. In particular, in our experiments we found that (*R*/*S*)-toloxatone and the related pyrrole analog (racemic mixture of **1a** and **1n**) were equipotent as MAO-A inhibitors, the latter being 6-fold less MAO-A selective than the former. Furthermore, the assays performed on the *R* (**1a**) and *S* (**1n**) enantiomers showed **1a** to possess the best activity and selectivity. Replacement of OH with other hydrophilic groups (amino, azido and dimethylamino) gave derivatives less potent and sometimes less selective than **1a**. On the contrary, *O*-alkylation of this compound afforded, i.e. (*R*)-5-methoxymethyl-3-(1*H*-pyrrol-1-yl)-2-oxazolidinone (**1b**), a MAO-A inhibitor endowed with very high potency and A-selectivity. In fact, compound **1b** ($K_{iMAO\text{-}A} = 4.9$ nM), is equipotent to befloxatone (*R*,*R* form) ($K_{iMAO\text{-}A} = 2.5$ nM), a new toloxatone analog, and it is characterized by very high selectivity towards the MAO-A isoenzyme (A-selectivity = 10,200, about 116-fold greater than that of befloxatone).

Compounds **2** and **3**, tested as anti-MAO agents, were more active against the A than against the B isoform of the enzyme (Table 4).

In compounds **2** and **3** the 2-oxazolidinone ring has been placed at C_2 and C_3 position of the pyrrole ring, respectively, while the pyrrole N_1 position remains free to be linked by groups with growing steric hindrance. Nevertheless, the introduction of such N_1 substituents decreases the MAO inhibitory activity of the compounds leading to derivatives less potent and selective than the **1** counterparts. This potency abatement is

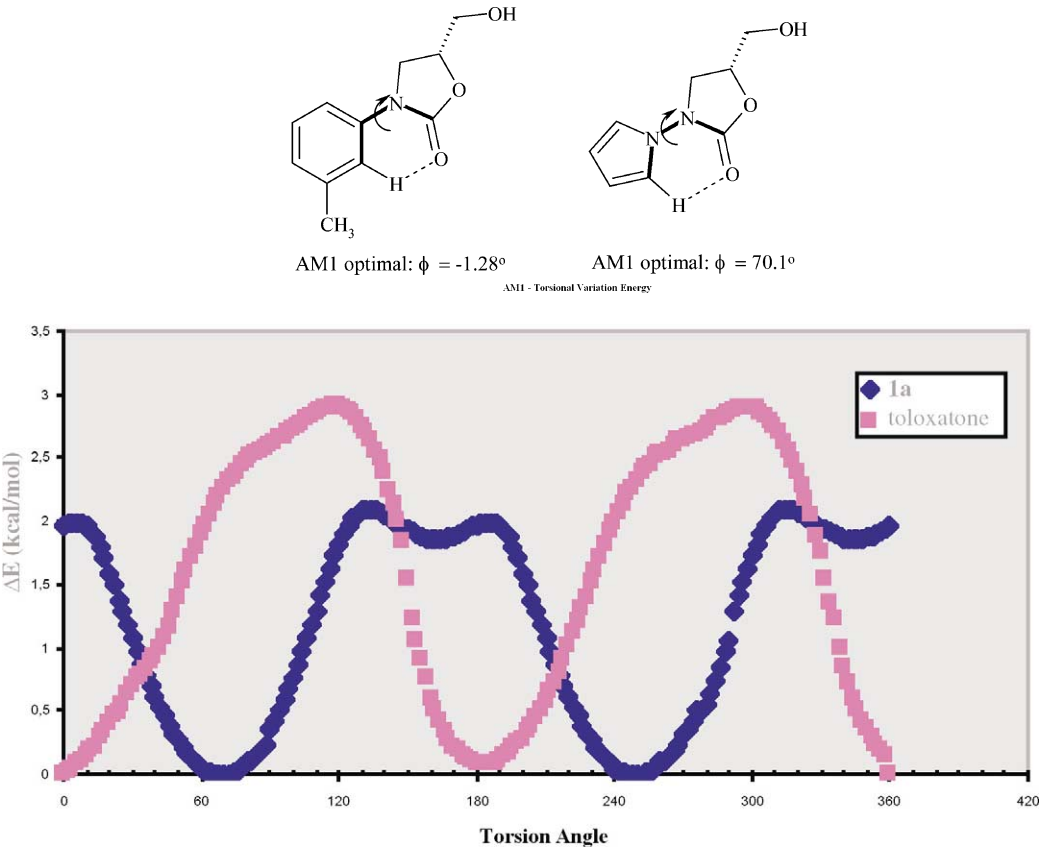


Fig. 7. AM1 calculations of optimal torsion angles of toloxatone (left) and **1a** (right).

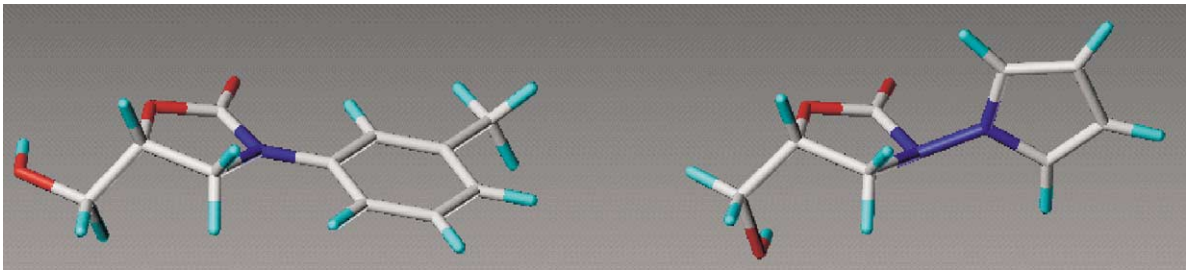


Fig. 8. Global minima conformations for toloxatone (left) and **1a** (right).

strictly dependent on the steric hindrance exerted by N_1-alkyl substituents: N_1-methyl-pyrrole derivatives represent the products belonging to the most active serie, followed by the N_1-ethyl and N_1-allyl analogues. N_1-Benzyl derivatives are practically inactive as anti-MAO agents, with the exception of the *R*-5-methylamino-3-(1-benzyl-1*H*-pyrrol-2-yl)-2-oxazolidinone (**2p**), which is endowed with both anti-MAO-A and anti-MAO-B activities ($K_{iMAO\text{-}A} = 0.14$ μM; $K_{iMAO\text{-}B} = 0.5$ μM). Among derivatives **2** and **3**, the most active compound resulted the *R*-5-azidomethyl-3-(1-methyl-1*H*-pyrrol-2-yl)-2-oxazolidinone **2c** (K_i against MAO-A = 0.004 μM; K_i against MAO-B = 4 μM; A-selectivity = 1000), being as potent as befloxatone and 10 times more A-selective.

4. Preliminary molecular modelling studies

X-ray diffraction-crystallographic studies coupled with conformational and electronic characterization (by the ab initio molecular orbital method) performed on toloxatone [30,31] showed that the drug is a planar molecule within the presence of an electron delocalization of both the oxazolidinone and phenyl rings. Such structural and electronic properties account for the existence of a charge-transfer complex between toloxatone and riboflavine and establish the mechanism of MAO-A reversible inhibition exerted by toloxatone.

Starting from these data, we performed conformational analysis on pyrrole analogue of toloxatone, **1a**, to

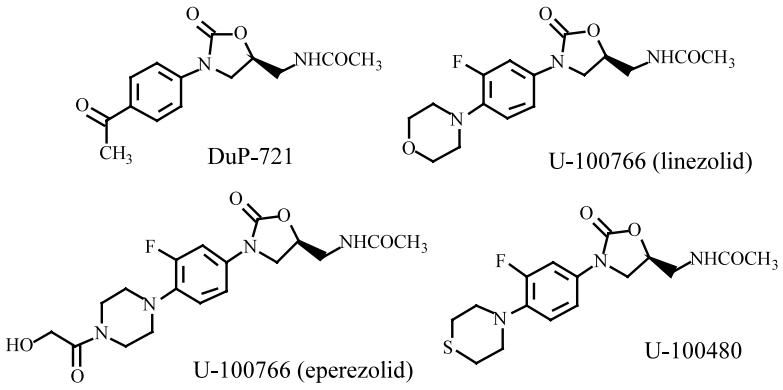
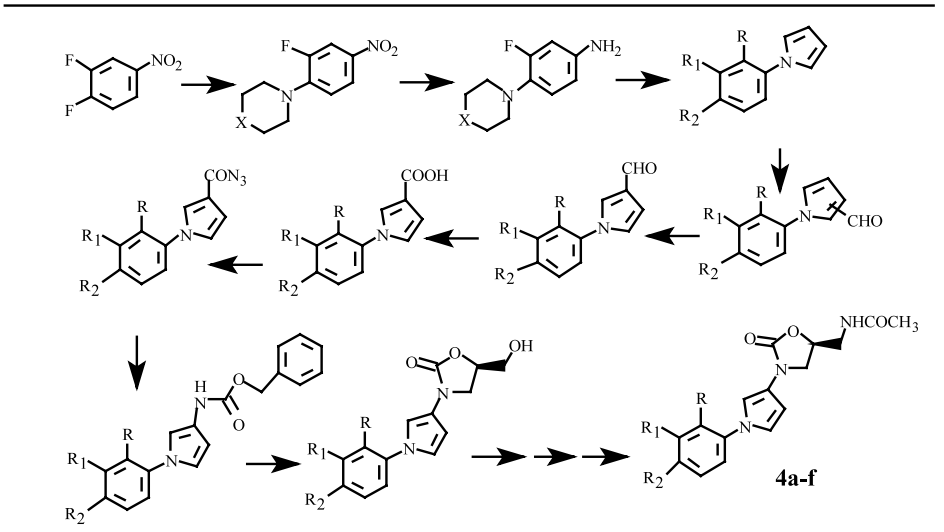


Fig. 9. Phenyl-oxazolidinones as antibacterial and antimycobacterial agents.



Scheme 3. Synthesis of 3-(1-aryl-1*H*-pyrrol-1-yl)-2-oxazolidinones **4a**–**f**.

verify if such co-planarity between pyrrole and oxazolidinone rings could exist also in our molecule. Surprisingly, we found that global minima conformations for toloxatone and **1a** (from MOPAC93 calculations) resulted quite different, being optimal torsion angles = −1.28° (toloxatone) and +70.1° (**1a**) (Figs. 7 and 8).

5. 3-(1-Aryl-1*H*-pyrrol-3-yl)-2-oxazolidinones (4) as antimycobacterial agents

After the discovery, in late 1984, of 5-acetamidomethyl-3-aryl-2-oxazolidinones as new synthetic antibacterial agents, the 3-aryl-2-oxazolidinone moiety has received much attention by the researchers. DuP 721, described by the DuPont group in 1987, was the first lead compound and clinical candidate active against gram-positive and -negative pathogens, as well as against *Mycobacterium tuberculosis*. In 1995 a team from the Upjohn Co. reported on the development of two *S*-5-acetamidomethyl-2-oxazolidinones, U-100592 and U-100766 (eperezolid and linezolid), which are now under clinical trials (the former) or approved by FDA (the latter) as novel potent and selective broad-spectrum antibacterial agents [40,41] (Fig. 9).

A great number of modifications were performed on various oxazolidinone-containing compounds to obtain antibacterial agents, having as unchanged moiety the 3-phenyl-2-oxazolidinone structure. Very little is reported about heterocyclic analogues of the above lead compounds.

Starting from these findings, we prepared pyrrolyl-oxazolidinones **4** showing an aryl moiety as substituent at the N_1 position of the pyrrole ring and carrying an hydroxymethyl, aminomethyl, and acetamidomethyl chains at the C_5 position of the 2-oxazolidinone nucleus to test as antibacterial and antimycobacterial agents (Scheme 3).

Table 5
Antimycobacterial activity of compounds **4**

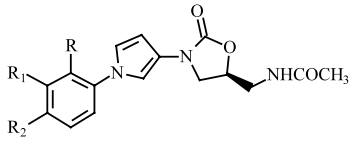
Compd.	MIC_{50} [a] (μM)			
	M. tuberculosis ATCC	*M. tuberculosis* clin. isol. 1104	MAC	*M. smegmatis*
4a	19	10.5	4	9.8
4b	3.6	nd	2.0	4.2
4c	12.9	9.4	4.6	14.5
4d	1.9	nd	1.4	10.3
4e	12.9	49	16.5	42.6
4f	9.8	12.2	5.8	34
linezolid	0.1	0.2	0.2	0.3
U-100480	0.1	0.5	0.7	0.5

[a] Minimum inhibitory concentration required to reduce the number of viable Mycobacteria by 50%, as determined by the MTT method.

Table 6
Antimycobacterial activity of compounds **4** against drug-resistant strains

Compd.	MIC_{50} [a] (μM)		
	M. tuberculosis ATCC 35820	*M. tuberculosis* ATCC 35828	*M. tuberculosis* ATCC 35837
4a	5.6	50.3	23.8
4b	1.1	10	7.0
4c	0.9	18.4	7.0
4d	0.8	21.5	13.5
4e	4.9	> 100	63.5
4f	nd	nd	nd
Linezolid	0.2	0.6	0.15
U-100480	0.006	0.1	0.06
Streptomycin	> 100		
Pyrazinamide		> 100	
Ethambutol			> 100

[a] Minimum inhibitory concentration required to reduce the number of viable Mycobacteria by 50%, as determined by the MTT method.



4a-f

a: R = R_1 = R_2 = H
b: R = F, R_1 = R_2 = H
c: R = R_2 =H, R_1 = F
d: R = R_1 = H, R_2 = F
e: R = H, R_1 = F, R_2 = morpholine
f: R = H, R_1 = F, R_2 = thiomorpholine

Such derivatives resulted devoid of antibacterial activity but endowed with an interesting antimycobacterial action against *M. tuberculosis*, *M. avium*, and *M. smegmatis*, and also against some *M. tuberculosis* strains resistant to known drugs (Tables 5 and 6).

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